Supplementary Information

Sensitive detection of transcription factors with Ag^+-stabilized self-assembly triplex DNA molecular switch

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Chemicals and materials

All oligonucleotides were synthesized and purified by Sangon (Shanghai, China). The sequences were shown Table 1. The purified recombinant NF-κB p50 protein was purchased from Cayman Chemical (Ann Arbor, MI, USA). HeLa nuclear extract and HeLa nuclear extract (TNF-α stimulated) were purchased from Active Motif (Carlsbad, CA, USA). Human thrombin, human immunoglobulin G (IgG) and bovine serum albumin were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All other chemicals (analytical grade) were obtained from standard reagent suppliers and used without further purification. All solutions were prepared using the ultrapure water that was obtained through a Millipore Milli-Q water purification system (>18.25 MΩ). All the single-stranded DNA stock solutions (100 μM) were prepared by solving them in ultrapure water and stored in the dark at -20 °C. The working solution of 2.5 μM was obtained by diluting the stock solution with 10 mM PBS buffer (pH 7.4). To obtain the 6-FAM-dsDNA, the corresponding complementary single-stranded DNA (DNA-1 and DNA-2) were mixed at the same molar ratios with the final concentration of 1.25 μM in the hybridization buffer (10 mM Tris−HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA). The mixture was heated at 95 °C for 5 minutes and then slowly cooled down to room temperature.

Measurement of fluorescence spectrum

2 μL 6-FAM-dsDNA (1.25 μM) was mixed with 10 μL BHQ-1-TFO (2.5 μM) and 2 μL $AgNO_3$ (500 μM) for 30 minutes at 37 °C in 10 mM PBS buffer solution (pH 7.4, 200 mM $NaNO_3$). For the detection of purified recombinant NF-κB p50, 2.5 μL purified recombinant NF-κB p50 (11 μM) were added to above system to incubate 10 minutes at 37 °C. For the detection of real samples, 2.5 μL TNF-α stimulated HeLa nuclear extracts (0.5 μg/μL) were added to above system to incubate 10 minutes at 37 °C. The final volume was 50 μL. The final concentrations were 50 nM of 6-FAM-dsDNA, 500 nM of BHQ-1-TFO, 20 μM of $AgNO_3$, 0.55 nM of purified recombinant NF-κB p50 and 25 ng/μL of TNF-α stimulated HeLa nuclear extract,

respectively. Finally, the fluorescence spectrums were recorded on Hitachi F-7000 spectrofluorophotometer (Hitachi, Japan). The excitation and emission wavelengths were set at 495 and 518 nm, respectively.

UV melting curves of the triplex DNA

10 μL 6-FAM-dsDNA (50 μM) was mixed with 5 μL BHQ-1-TFO (100 μM) and 20 μL $AgNO_3$ (500 μM) for 30 minutes at 37 °C in 10 mM PBS buffer solution (pH 7.4, 200 mM $NaNO_3$). The final volume was 500 μL. The final concentrations were 1 μM of 6-FAM-dsDNA, 1 μM of BHQ-1-TFO and 20 μM of $AgNO_3$, respectively.

The melting temperatures of the triplex DNAs were determined by monitoring the absorbance intensities at 260 nm at different temperatures. The absorbance intensities were then plotted as a function of the temperature ranging from 25 to 80 °C. The values of melting temperatures of the triplex DNAs were obtained from the transition midpoint of the melting curves. UV absorption spectras were recorded on UV-2450 spectrophotometer (Shimadzu, Japan).

Results

The recognition and detection of NF-κB p50 depended greatly on the stability of the triplex DNA. Therefore, the influencing factors of stability, such as the concentration of Ag^+, the length of the BHQ-1-TFO, the concentration of the BHQ-1-TFO and pH were investigated.

The first influencing factor was the Ag^+ concentration (see Fig. S1, ESI†). ΔF increased along with the increase of Ag^+ concentration and reached a plateau after 20 μM. This phenomenon demonstrated that Ag^+ indeed improved the stability of triplex DNA, which was consistent with the previous report.[1] As a result, 20 uM of Ag^+ was chosen for further experiments. Moreover, as shown in Fig. S5, compared with original melting temperature of the triplex DNA (Tm=33 °C) in the absence of Ag^+, the raised melting temperature (Tm2=57 °C) in the presence of Ag^+ further substantiated the stabilization effect of Ag^+ on the triplex DNA.

The second influencing factor was the length of the BHQ-1-TFO (see Fig. S2, ESI†). The affinity of BHQ-1-TFO for 6-FAM-dsDNA was enhanced with the increase of the length of the BHQ-1-TFO from 12-mer to 15-mer, which resulted in

the increase of the amounts of triplex DNAs and the decrease of F0. However, when the length of the BHQ-1-TFO reached 18-mer, the increased electrostatic repulsion between 6-FAM-dsDNA and BHQ-1-TFO weakened the affinity of BHQ-1-TFO for 6-FAM-dsDNA, which resulted in the decrease of the amounts of triplex DNAs and the increase of F0. In other word, F0 had a minimum value with 15-mer BHQ-1-TFO. Because the affinity of NF-κB p50 for dsDNA is much stronger than that of TFO for dsDNA[2,3], it was very easy for NF-κB p50 to displace BHQ-1-TFO (from 12-mer to 18-mer). Almost the same fluorescence intensity (F) could be observed with all three triplex DNA probes upon NF-κB p50 addition. Therefore, ΔF had a maximum value with 15-mer BHQ-1-TFO. We chose 15-mer BHQ-1-TFO for further experiments. In addition, as shown in Fig. S5, the melting temperatures (Tm) of three triplex DNAs were 47 °C (Tm1= 47 °C when using 12-mer BHQ-1-TFO), 57 °C (Tm2= 57 °C when using 15-mer BHQ-1-TFO) and 52 °C (Tm3=52 °C when using 18-mer BHQ-1-TFO), respectively. Tm had a maximum value with 15-mer BHQ-1-TFO. Compared with Tm2, the lower Tm3 attributed to the reduction of the amount of triplex DNAs that resulted from the increased electrostatic repulsion between TFO and dsDNA. This result further substantiated that in the fluorescence analysis the high F0 when using the 18-mer TFO was indeed due to the increased electrostatic repulsion between TFO and dsDNA.

The third influencing factor was the concentration of BHQ-1-TFO (see Fig. S3, ESI†). The ΔF increased along with the increase of the BHQ-1-TFO concentration and reached a plateau after 500 nM. Therefore, 500 nM BHQ-1-TFO was selected for the reasearch.

The fourth influencing factor was pH (see Fig. S4, ESI†). The ΔF increased with the pH value over the range of 6.0-7.4 and reached the maximum at pH 7.4. However, the ΔF decreased dramatically when the pH value was higher than 7.4. The reason is that the alkaline condition was unsuitable for the formation of the triplex DNA (Ag^{+}-STDMS) because of the formation of the silver oxide precipitation. So, pH 7.4 was the optimum condition for the research.

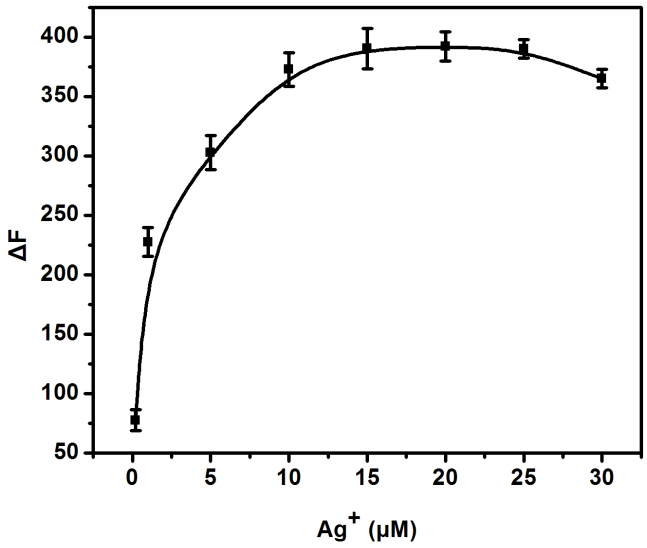

Fig. S1 The effect of Ag^+ on the ΔF.

Experimental conditions: 10 mM of PBS (pH 7.4), 200 mM of $NaNO_3$, 50 nM 6-FAM-dsDNA, 500 nM BHQ-1-TFO (15-mer), 0.55 nM of NF-κB p50, 30 minutes of self-assembly at 37 °C, 10 minutes of displacement at 37 °C.

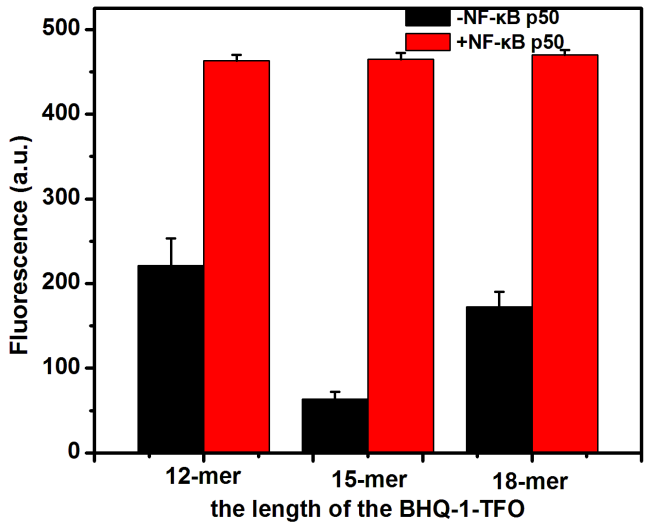

Fig. S2 The effect of the length of the BHQ-1-TFO on the ΔF.

Experimental conditions: 10 mM of PBS (pH 7.4), 200 mM of $NaNO_3$, 50 nM 6-FAM-dsDNA, 500 nM BHQ-1-TFO, 20 μM of Ag^+, 0.55 nM of NF-κB p50, 30 minutes of self-assembly at 37 °C, 10 minutes of displacement at 37 °C.

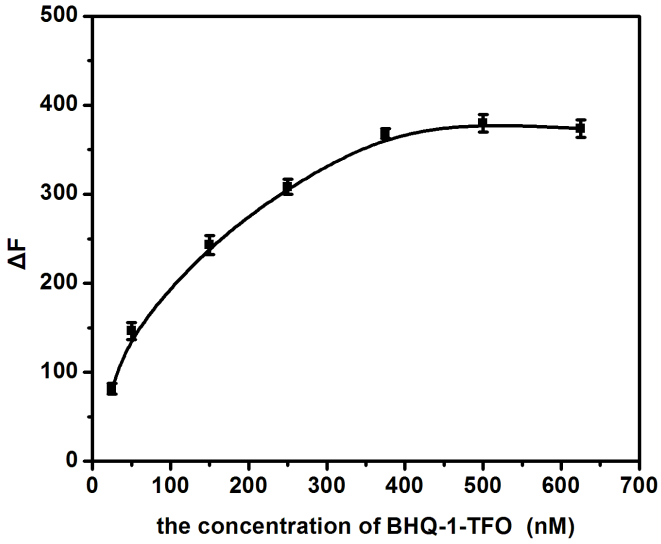

Fig. S3 The effect of the concentration of BHQ-1-TFO (15-mer) on the ΔF. Experimental conditions: 10 mM of PBS (pH 7.4), 200 mM of $NaNO_3$, 50 nM 6-FAM-dsDNA, 20 μM of Ag^+, 0.55 nM of NF-κB p50, 30 minutes of self-assembly at 37 °C, 10 minutes of displacement at 37 °C.

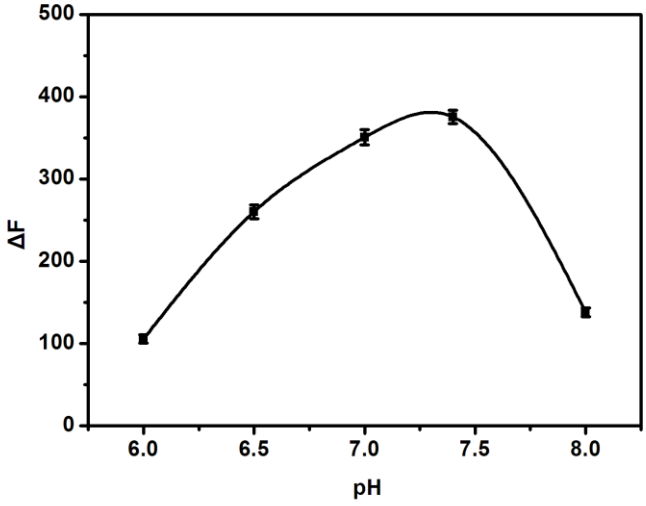

Fig. S4 The effect of pH on the ΔF. Experimental conditions: 10 mM of PBS, 200 mM of $NaNO_3$, 50 nM 6-FAM-dsDNA, 500 nM BHQ-1-TFO (15-mer), 20 μM of Ag^+, 0.55 nM of NF-κB p50, 30 minutes of self-assembly at 37 °C, 10 minutes of displacement at 37 °C.

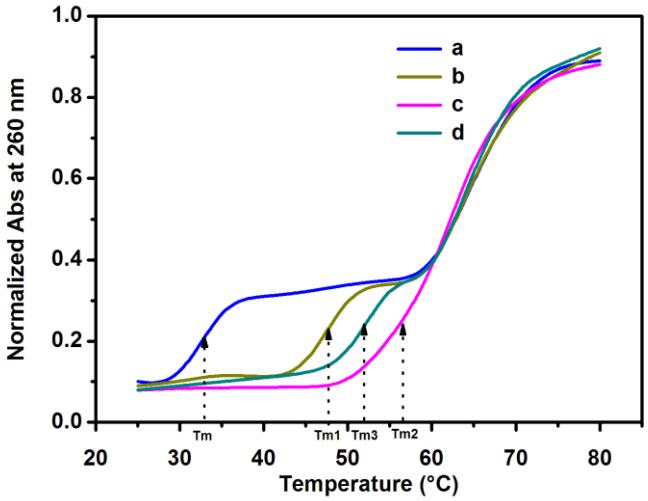

Fig. S5 UV melting curves of triplex DNA obtained in the presence of various amounts of Ag^+ and different length of the BHQ-1-TFO.

(a) 6-FAM-dsDNA + BHQ -1-TFO (15-mer), (b) 6-FAM-dsDNA + BHQ-1-TFO (12-mer) + Ag^+, (c) 6-FAM-dsDNA + BHQ-1-TFO (15-mer) + Ag^+, (d) 6-FAM-dsDNA + BHQ-1-TFO (18-mer) + Ag^+.

Experimental conditions: 10 mM of PBS (pH 7.4), 200 mM of $NaNO_3$, 1 μM 6-FAM-dsDNA, 1 μM BHQ-1-TFO, 20 μM of Ag^+, 30 minutes of self-assembly at 37 °C.

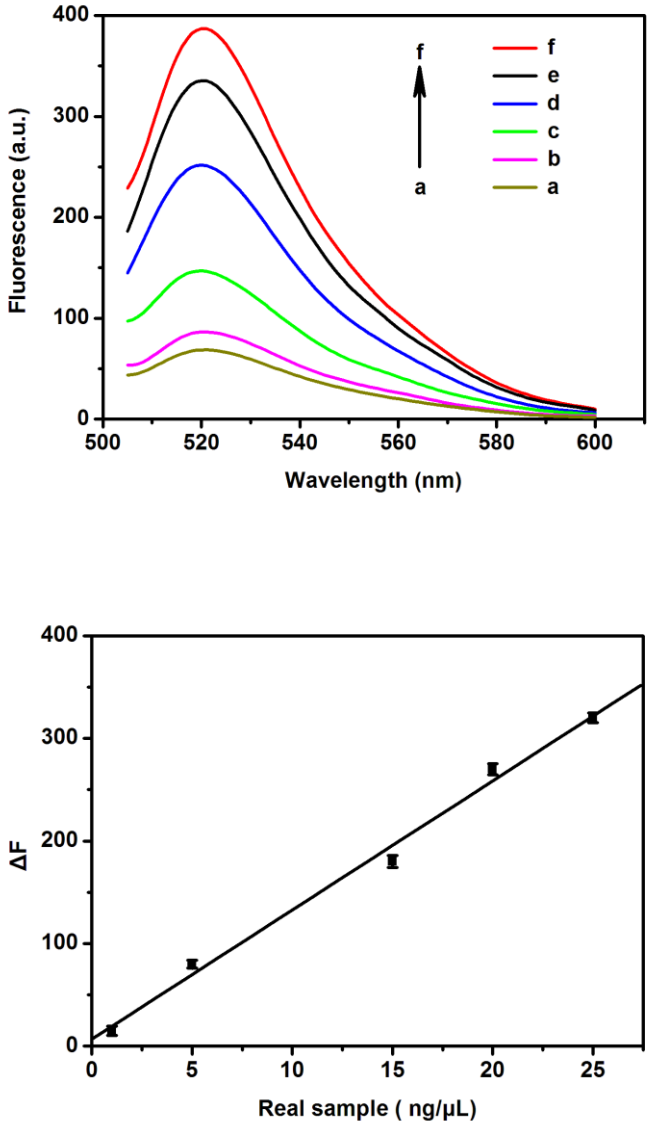

Fig. S6 The fluorescence spectrums with increasing concentration of TNF-α-treated nuclear extracts (above) and the relationship between fluorescence intensity and the concentration of TNF-α-treated nuclear extracts (below). The error bars showed the standard deviation of three replicate determinations. Experimental conditions: 10 mM of PBS (pH 7.4), 200 mM of $NaNO_3$, 50 nM of 6-FAM-dsDNA, 500 nM of BHQ-1-TFO (15-mer), 20 μM of Ag^+, target (a to f): 0, 1, 5, 15, 20 and 25 ng/μL, respectively. 30 minutes of self-assembly at 37 °C, 10 minutes of displacement at 37 °C. The excitation and emission wavelengths were set at 495 nm and 518 nm, respectively.

Table S1. Comparison of strategies for detection of NF-κB p50.

Strategy	Limit of detection
ref 4	1 nM
ref 5	1 nM
ref 6	5 nM
ref 7	0.1 nM
this work	0.025 nM

Reference

1 T. Ihara, T. Ishii, N. Araki, A. W. Wilson and A. Jyo, *J. Am. Chem. Soc.*, 2009, **131**, 3826–3827.

2 M. B. Urban, P. A. Baeuerle, *genes & development*, 1990, **4**, 1975-1984.

3 L. J. Maher, III, P. B. Dervan, and B. J. Wold, *Biochemistry*, 1990, **29**, 8820-8826.

4 A. Vallee-Belisle, A. J. Bonham, N. O. Reich, F. Ricci and K. W. Plaxco, *J. Am. Chem. Soc.*, 2011, **133**, 13836–13839.

5 J. J. Liu, X. R. Song, Y. W. Wang, G. N. Chen and H. H. Yang, *Nanoscale*, 2012, **4**, 3655–3659.

6 J. K. Wang, T. X. Li, X. Y. Guo and Z. H. Lu, *Nucleic Acids Res*., 2005, **33**, e23.

7 X. F. Liu, L. Ouyang, X. H. Cai, Y. Q. Huang, X. L. Feng, Q. L. Fan, W. Huang, *Biosens. Bioelectron*., 2013, **41**, 218−224.